CAN GENOMICS OF DRY MATTER INTAKE IN TRANSITION COWS IMPROVE HEALTH AND FERTILITY?

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INTRODUCTION

The periparturient transition period in dairy cows encompasses the 6-8 weeks of late pregnancy and early lactation. Parturition results in an abrupt metabolic shift from nutrient accrual (fetus and body reserves) to rapid mobilization of lipid and protein stores for energy metabolism in support of milk production. The cascade of metabolic and physiological changes in the peripartal cow affect productivity, health and future reproductive performance.

In high genetic merit cows, feed intake and energy balance may begin decreasing prepartum. In turn, lower pre-calving dry matter intake (DMI) has been implicated as a risk factor for ketosis and metritis after calving. The onset of lactation after calving is associated with a prolonged period of negative energy balance (NEB) during which energy intake lags behind the energy requirements of rapidly increasing milk production. Lower DMI also affects subsequent reproductive function in that cows with lower voluntary intakes during the final weeks of pregnancy and greater NEB post-calving express delays in their resumption of estrous cycles in early lactation. Thus, levels of energy intake, re-initiation of ovarian activity in advance of the breeding period, and health status are intertwined in transition cows.

During the past decade, adoption of genomic selection in breeding programs by the major dairy-producing countries has led to significant changes in the worldwide dairy industry. When added to the routine evaluation for milk production and other economic traits, genomic selection has markedly accelerated genetic gains. Since sufficient DMI is important for high-producing cows to maintain health and to cover the demands of milk production, interest has increased for genomic prediction of DMI in dairy cattle within overall breeding goals. The purpose of this paper is to explore current information and future potential on the genomics of DMI in relation to successful transition from pregnancy to early lactation.

Prepartum Differences in DMI Associated with Cow Health

Metabolic adaptations in the periparturient cow are both dynamic and complex with the conditions changing daily. An emerging issue is that the pattern and timing of decreased DMI before calving is predictive of upcoming health disorders and perturbations in liver function. Cows that developed metritis (Huzzey et al., 2007) or ketosis (Goldhawk et al., 2009) after calving had lower daily DMI during the two weeks

prior to calving and spent less time feeding. Thus, changes in animal behavior and DMI occurred prior to calving and before any evidence of pathology post-calving.

In cows diagnosed with uterine infection (metritis), associated increases after calving in plasma haptoglobin, an acute phase protein, have been reported (Huzzey et al., 2009; Schneider et al., 2013). Haptoglobin is produced by the liver and is a marker of hepatic metabolic disturbance in response, for example, to inflammation of the uterus and the resultant secretion of cytokines. Cytokines are proteins secreted by white blood cells and tissues during inflammation as a response to irritation or injury caused by infection or other damage (Loor et al., 2013). The various cytokines can be classified as being pro-inflammatory cytokines (*e.g.* IL-6 and TNFα) or anti-inflammatory cytokines (*e.g.* IL-10). In the liver, pro-inflammatory cytokines rapidly promote the secretion into blood of positive acute phase proteins *e.g.* haptoglobin, whereas, the production and release of other blood proteins such as albumin decreases *i.e.* albumin is a negative acute phase protein (Schneider et al., 2013 and **Figure 1**).

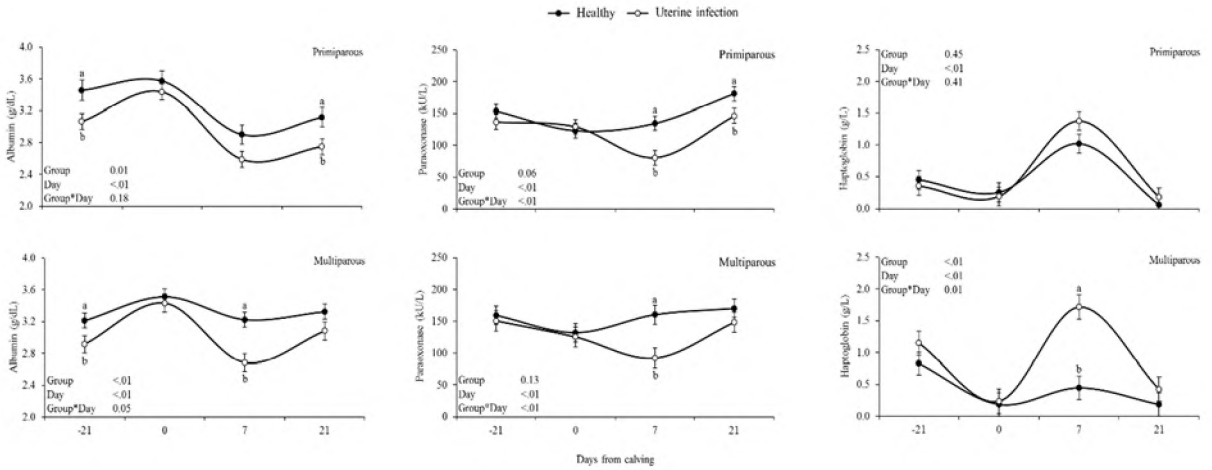

Figure 1. Serum concentrations of albumin (g/dL), paraoxonase activity (KU/L), and haptoglobin (g/L) in healthy cows or cows diagnosed with metritis during the transition period. Different letters indicate differences between groups.

A response to inflammation in postpartum cows can be monitored in terms of both positive and negative acute phase proteins and associated changes in other blood metabolites that allows for calculation of a liver functionality index (LFI; (Trevisi et al., 2012). Low LFI values are indicative of a high inflammatory response and increased pro-inflammatory cytokine release. Low LFI cows were much more likely to encounter disease or health problems after calving as well as having lower DMI and milk production. In cows that develop metritis after calving there is evidence of disturbance in liver function (*e.g.* acute phase responses - ↓ albumin and ↑ ceruloplasmin) already occurring during the last 3 weeks prepartum (Schneider et al., 2013; Trevisi et al., 2012).

The periparturient period is characterized by a sudden increase in energy requirements for lactation superimposed on declining voluntary DMI and this results in NEB. An important adaptation to NEB during the transition to early lactation is the

mobilization/lipolysis of fat from body adipose stores with release of non-esterified fatty acids (NEFA) into the circulation. NEFA provides an important source of energy during early lactation when the majority of available glucose is being spared for lactose synthesis in the mammary glands. However, continuous or excessive lipolysis promotes conversion of NEFA into liver triacylglycerol's that can negatively impact hepatic glucose production. The consistent responses in liver to lipid infiltration are reduction in the expression of genes and proteins associated with ATP production and up-regulation of markers of inflammation (*e.g.* IL-6; (Loor et al., 2007; Loor, 2010).

As described above, inflammation during the periparturient period has emerged as an important aspect of transition cow biology and an excellent review has recently appeared (Bradford et al., 2015). The presence of an inflammatory state in the postpartum period has been documented and the acute phase response, a key secondary response to inflammation, has been well established. The acute phase protein haptoglobin was shown to be elevated in plasma around calving, even in cows that were apparently healthy, but cows with health problems or calving difficulties had higher concentrations (Qu et al., 2014; Schneider et al., 2013 -- see figure1). Elevated haptoglobin levels have been associated with enhanced innate immune responses in white blood cells *i.e.* a systemic inflammatory state (Nightingale et al., 2015), however, the postpartum inflammatory state in many cows is low-grade without the classical signs of inflammation. Bradford et al. (2015) refer to this latter situation as subacute inflammation or metabolic inflammation that is associated with tissue malfunction *e.g.* liver function. In the postpartum period, exposure to high concentrations of fatty acids can disrupt intra-cellular endoplasmic reticulum (ER) membranes in the liver and cause a stress response (Ringseis et al., 2015). Lipid peroxides are also potential mediators that link elevated NEFA to hepatic inflammation. Enhanced peroxisomal oxidation of fatty acids contributes reactive oxygen species (ROS) that increase lipid peroxide formation with the potential for local cell and tissue injury.

Peripartum Changes in DMI Associated with Reproductive Performance

By way of various metabolic factors and hormonal interactions, NEB shifts the course of ovarian activity early postpartum and strongly influences the resumption of ovulatory ovarian cycles *i.e.* postpartum interval in days to first ovulation. With regard to fertility to AI during the breeding period, there is a strong positive association between early commencement of ovulatory cycles and pregnancy during lactation (Butler, 2000; Galvao et al., 2010). Following calving, ovarian follicle development and function were monitored for comparison, retrospectively with differences in prepartum DMI, NEB, and metabolic conditions. Cows that achieved full follicular function and successfully ovulated by 3 weeks of lactation had consistently higher prepartum DMI in the last 3-4 weeks before calving, their energy balance was higher (albeit still negative postpartum), and blood NEFA concentrations were lower as compared with cows that developed large nonovulatory follicles and had delayed first ovulation (Butler et al., 2006; Cheong et al., 2016) **See figure 2**. Overall, NEB is minimized in cows that maintain higher DMI until the day of calving and then rapidly increase their intakes over the first several weeks of lactation.

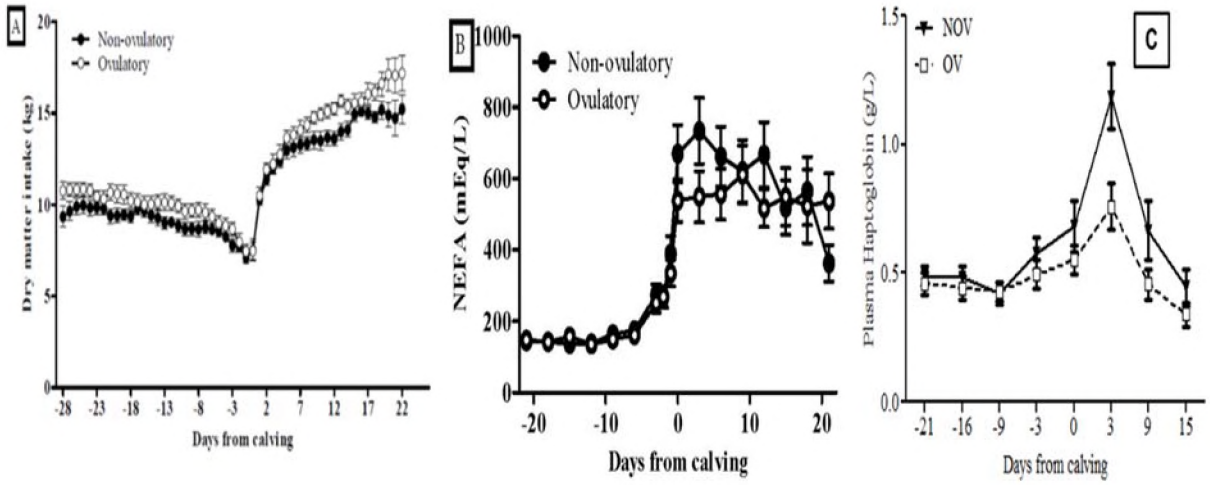

Figure 2. Comparison of the patterns of DMI (kg/d) and plasma concentrations of NEFA (μEq/L) and haptoglobin (g/L) during the transition period in dairy cows with postpartum ovulatory *vs.* nonovulatory dominant follicles. DMI was consistently higher and plasma levels of NEFA and haptoglobin were lower in cows having large follicles that successfully ovulated within 3 weeks after calving.

Increases in blood NEFA concentrations prior to parturition provide a monitoring tool to assess future impacts on reproduction. In a large field study involving 100 dairy herds, cows with NEFA ≥ 0.27 mEq/L in plasma during the week before calving subsequently had 19% lower probability for pregnancy after the start of breeding (Ospina et al., 2010). Cows with greater loss of body condition score loss associated with NEB in early lactation have lower fertility later during the breeding period (Santos et al., 2009).

As presented earlier, the periparturient period in cows is associated with reduced DMI, increased mobilization of NEFA from adipose tissue and a variable degree of liver acute phase response. A strong acute phase response to systemic inflammation characterized by an activated immune system and cytokine response *e.g.* high plasma concentrations of haptoglobin and TNFα, respectively, resulted in impaired reproductive efficiency and longer delays for cows to conceive during lactation (Nightingale et al., 2015). Higher plasma haptoglobin concentrations were also present in cows that developed nonovulatory large dominant follicles after calving (**see figure 2C**).

How might postpartum inflammatory conditions in conjunction with NEB exert carryover effects on fertility in dairy cows? A very interesting and comprehensive report involving large numbers of cows appeared recently (Ribeiro et al., 2016). Cows that were diagnosed with any inflammatory disease before AI had reduced pregnancy rate/AI; **either uterine disease or nonuterine disease** reduced pregnancy rate/AI and their effects were additive. Most interestingly, the occurrence of disease at <u>preantral **or** antral stages</u> of follicle/oocyte development had similar detrimental effects on pregnancy rate. This indicates that the carryover effects of inflammation can last longer than 12 weeks *i.e.* the estimated time period required for ovulatory follicle development in cows. Thus,

the authors concluded that reduced oocyte competence is the likely reason for the long term detrimental carryover effects of postpartum inflammation.

Genomic Analyses for Associations with DMI in Lactating Dairy Cows

DMI in dairy cows has moderate heritability and, thus, provides a potentially useful target for genetic selection (Liinamo et al., 2012; Spurlock et al., 2012). Identifying genetically superior animals for DMI is difficult and requires having many animals with phenotypic and genotypic data. As an example of one research approach, the initial goal is to identify genomic regions (areas of chromosomes) significantly associated with DMI. DNA samples from individual cows can be genotyped using beadchips with 50K or 770K single nucleotide polymorphism (SNP) markers for comparison with phenotype information collected on DMI during specific stages of lactation. Genome-wide association studies (GWAS) are then conducted to statistically link markers or groups of markers on specific chromosomes with changes in DMI. The basic approach in GWAS is to evaluate the association between each genotyped marker and a phenotype of interest evaluated in a group of individuals. The results can be depicted in Manhattan plots showing markers or peaks of markers that meet or exceed the statistical criteria for significance (threshold line). An example of a Manhattan plot for DMI at 30 days of lactation is shown in **figure 3A** (Tetens et al., 2014).

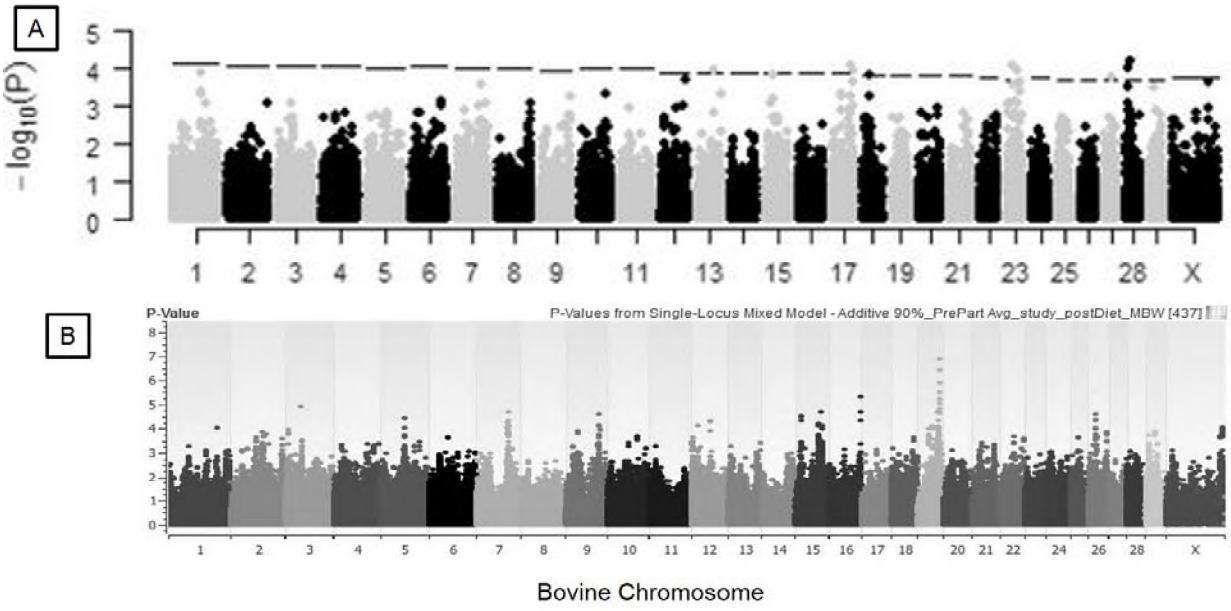

Figure 3. Results of genome-wide association studies (GWAS) depicted in Manhattan plots with chromosomal location of markers associated with DMI. **A)** Data for 30 days of lactation (Tetens et al., 2014). The horizontal line indicates the threshold for chromosome-wise significance. **B)** Data for the first 2 weeks of lactation using 90% as the cut-off threshold for daily energy intake as % of required (Butler et al., unpublished).

In the GWAS study shown in figure 3A, DMI was monitored in primiparous cows during intervals from 11-80 DIM. Significant markers of DMI were found on 8 of the 30 bovine chromosomes (#13, 15, 17, 23, 25, 27, 28, and 29). Using a similar approach in research trials at Cornell University, a GWAS analysis was conducted for a phenotypic variable associated with DMI: *daily dietary energy intake as a % of total energy required* during the first 2 weeks of lactation. This variable was chosen rather than NEB (Liinamo et al., 2012) in order to identify individual cows that best met their energy requirements *via* higher voluntary feed intake and requiring less adipose lipid mobilization. Using a cut-off level of 90% for the high *versus* lower intake cows, the Manhattan plot for genomic areas associated with the phenotype is shown in **figure 3B** (Butler, unpublished). All cows were multiparous and remained healthy postpartum. The 8 chromosomes having the highest concentrations of markers associated with *energy intake as a % of requirement* were: #3, 5, 7, 12, 15, 16, 19, and 26. Obviously, there appears to be little concordance in the genomic regions identified for association with DMI in figure 3. A previous study suggested that lactating cows express their genetic potential for feed intake and energy utilization most clearly between weeks 2 to 10 of lactation (Liinamo et al., 2012) and this has focused our recent research attention.

A second approach for genomic analysis related to DMI in lactating cows is to identify specific candidate genes in physiological pathways relevant to DMI. Researchers may study genes that have shown effects on DMI in previous studies. The objective is to compare the phenotype data among animals with their genotypic polymorphisms *i.e.* do cows having the different alleles (*e.g.* AA, AB, or BB) in the gene show differences in their DMI performance? There are ample examples of differences in the DNA code resulting in differences in the amino acid sequence of the protein product from the gene that may alter phenotype. One example of the candidate gene approach was an earlier study reporting associations between a SNP in the growth hormone receptor gene (GHR) and differences in DMI in dairy cattle (Banos et al., 2008).

GWAS for DMI can also identify potential candidate genes for future research. Once the SNP markers associated with DMI are mapped to specific genomic regions on the various chromosomes, public databases containing information on the bovine genome can be interrogated to find genes in close proximity to the markers. Biological information on any effects of these genes that may be associated with regulation of the phenotype is then assembled for further studies and confirmation. Veerkamp et al. (2012) identified candidate genes for feed intake linked to insulin, cellular growth factors and tryptophan. In another example, adrenergic receptor and leptin genes were identified as candidates to affect DMI from among a list of many other genes in the same chromosome region (Hardie et al., 2017). In the recent Cornell studies, the SLC37A1 gene was highly associated with DMI in early lactation. The protein product of this gene translocates glucose-6-phosphate from the cytoplasm into the lumen of the ER for hydrolysis into glucose by another ER membrane protein. This gene is a member of the solute carrier 37 gene family. Although difficult and expensive, further research will continue to investigate the manner by which multiple genes and their genotype combinations can better predict effects on DMI.

In addition to many research studies investigating the genomic associations directly with DMI in lactating cows, there has also been interest regarding dietary energy utilization and feed efficiency. In a recent study, gross feed efficiency (GFE), defined as the ratio of total energy corrected milk to total DMI, was measured during the first 150 DIM along with energy balance (Spurlock et al., 2012). The genetic correlation between GFE and energy balance ranged from -0.73 to -0.99, indicating that selection for more dietary energy efficient cows would favor a lower energy status. This negative relationship between GFE and energy balance means that higher efficiency cows are deriving more of their energy requirement for milk production from body tissues. Indeed, another study showed that cows who had higher feed efficiency during lactation had greater days open (lower fertility) -- the genetic correlation between GFE and days open was 0.53 (Vallimont et al., 2013). Thus, results from both studies indicate that selection for improved feed efficiency must be carefully considered in order to avoid potential negative consequences on health and fertility associated with greater NEB in early lactation.

CONCLUSIONS

1. During the periparturient period in cows, changes in DMI, health status, and re-initiation of ovarian activity are intertwined.
2. The periparturient period in cows is characterized by reduced DMI, increased mobilization of NEFA due to NEB, and a variable degree of liver metabolic disturbance/acute phase response.
3. Differences in peripartum DMI among cows are associated with differing effects on ovarian function and subsequent reproductive performance.
4. Genomic analysis has demonstrated that DMI is a complex trait associated with many genomic regions and individual genes.
5. Genomic tools hold promise for better genetic selection for DMI in dairy cows to improve health and reproduction by minimizing NEB.

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